**Must-Knows: Unit 10 (Gene Regulation and Biotechnology)**

Ms. Ottolini, AP Biology

**Test Format:** 18 multiple choice questions, 1 short answer question

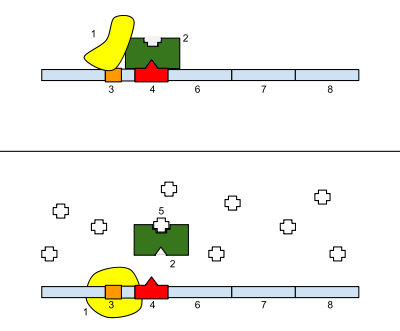
**Topic #1: Gene Regulation**

**Learning Target #1:** I can identify the purpose of gene regulation in prokaryotic and eukaryotic cells.

**Learning Target #2:** I can explain how inducible and repressible operons are regulated in prokaryotic cells.

**Learning Target #3:** I can compare / contrast the different levels of gene regulation in eukaryotic cells.

**Topic #2: Mitosis**

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With regard to the operon pictured to the right, the image on top shows the operon in its normal state, and the image on the bottom shows the operon in the presence of molecule #5 (looks like a + sign).

1) Identify the different parts of the picture. Your options are repressor, promoter, genes of the operon, operator, RNA polymerase, and inducer.

1. RNA polymerase
2. Repressor
3. Promoter
4. Operator
5. Inducer

6-8. Genes of the operon

2) What type of operon is shown—inducible or repressible—and how do you know?

This is an inducible operon. In its normal state it is turned off (RNA polymerase cannot bind to the promoter correctly to begin transcription of the genes of the operon). In the presence of the inducer molecule, the operon can be turned on.

3) What is the role of molecule #5 in regulating the operon shown above?

In the presence of the inducer molecule (#5), the repressor is inactivated and cannot bind to the operator, allowing RNA polymerase to bind correctly to the promoter and begin transcription of the genes of the operon.

4) Why is a catabolic operon (one that contains genes for enzymes used to break down molecules) usually an inducible operon?

It is usually turned off but can be turned on in the presence of a particular molecule in the environment that must be broken down (ex: lactose sugar).

5) Why is an anabolic operon (one that contaisn genes for enzymes used to build molecules) usually a repressibel operon?

It is usually turned on because it is used to synthesize an essential molecule (ex: tryptophan), but it can be turned off if the essential molecule is present in high concentrations.

6) Let’s say methyl groups are added to the DNA of the gene coding for human growth hormone. How will this affect the amount of human growth hormone produced?

Methyl groups cause DNA to coil tightly, preventing RNA polymerase from binding to the promoter to begin transcription of the gene. This will decrease the amount of gene expression and thus the amount of human growth hormone produced.

7) Let’s say acetyl groups are added to the histone proteins that interact wth the DNA of the gene coding for human growth hormone. How will this affect the amount of human growth hormone produced?

Acetyl groups cause DNA to coil less tightly, allowing RNA polymerase to bind to the promoter to begin transcription of the gene. This will increase the amount of gene expression and thus the amount of human growth hormone produced.

8) How is the regulation of gene expression different for prokaryotic cells vs. eukaryotic cells?

Prokaryotic cells only regulate gene expression at the transcription level (either preventing or facilitating the binding of RNA polymerase to the promoter of an operon). Eukaryotic cells regulate gene expression at many different levels of protein synthesis—including DNA structure, transcription, post-transcription (mRNA processing), translation, and post-translation.

9) How can changes at the level of mRNA processing (after transcription) produce totally different proteins (ex: variations of the different antibody proteins that are targeted to attack specific bacteria or viruses?

Splicing (i.e. cutting) out the introns in different ways / combinations can produce multiple proteins from the same gene.

**Topic #2: Biotechnology A**

**Learning Target #4:** I can describe the purpose and methods of gel electrophoresis and analyze electrophoresis results.

**Learning Target #5:** I can describe the purpose and methods of polymerase chain reaction (PCR).

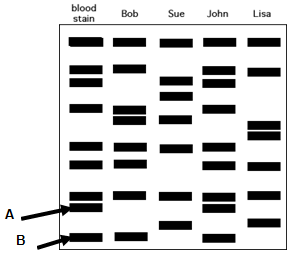
**Learning Target #5:** I can compare the types of DNA mutations and their effects on the resulting protein.

10) Explain the purpose of each of the three factors in polymerase chain reaction (PCR).

1. Heat: “Denatures” (i.e. separates) the two strands of the DNA double helix by breaking the hydrogen bonds between complementary nitrogen bases.
2. Primers: Short sequences of DNA nucleotides that anneal (stick) to the beginning of the target sequence and tell Taq polymerase where to begin replicating the target sequence.
3. Taq polymerase: Brings in free nucleotides to match up with the nucleotides of the target sequence to create copies of the target sequence.

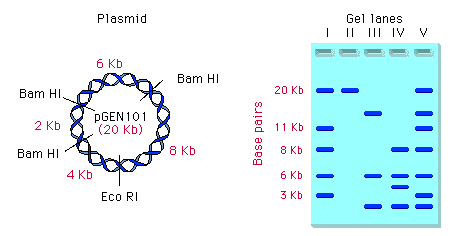
11) Which person in the DNA fingerprint shown to the right—Bob, Sue, John, or Lisa—matches the blood stain DNA? How do you know?

John ; his pattern of restriction fragments (bands) matches with the blood stain pattern.

12) If the “wells” of the gel are located up at the top, which DNA fragment is larger—A or B? How do you know?

Fragment A is larger because it migrates more slowly towards the far end (the positive end) of the gel.

Below is a plasmid with restriction sites for BamHI and EcoRI. Several restriction digests were done using these two enzymes either alone or in combination. Use the figure to answer questions 13-15

  
**Hint:** Begin by determining the number and size of the fragments produced with each enzyme. "kb" stands for kilobases, or thousands of base pairs.

13) Which lane shows a digest with BamHI only?

If only BamHI is able to make “cuts” on the plasmid, it will produce fragments of the following sizes – 2 Kb, 6 Kb, and 12 Kb. Therefore, Lane III shows a digest with BamHI only.

14) Which lane shows a digest with EcoRI only?

If only EcoRI is able to make “cuts” on the plasmid, it will produce a single fragment with a length of 20 kb. Therefore, Lane II shows a digest with EcoRI only.

15) Which lane shows a digest with both BamHI and EcoRI?

If both BamHI and EcoRI are able to make “cuts” on the plasmid, it will produce fragments of the following sizes – 2 Kb, 6 Kb, 8 Kb, and 4 Kb. Therefore, Lane IV shows a digest with both BamHI and EcoRI.

**Topic #3: Biotechnology B**

**Learning Target #6:** I can describe the purpose and methods of bacterial transformation and analyze bacterial transformation results.

**Learning Target #7:** I can provide examples of the practical uses of biotechnology.

In a lab experiment that WORKED (clearly not Ms. Ottolini’s lab), scientists transformed *E. coli* bacteria with a plasmid containing the gene for ampicillin resistance (ampR) and the gene to enable the bacterium to glow (pGlo). The pGlo gene is typically turned off but can be turned on in the presence of the sugar arabinose (ara). The scientists attempted to grow cultures of this transformed bacteria in three conditions—plain LB agar (bacteria food), LB / amp, and LB / amp / ara. They then attempted to grow cultures of untransformed bacteria (lacking the plasmid) in the same three conditions. The table below summarizes all the treatment groups.

|  |  |  |  |
| --- | --- | --- | --- |
|  | LB | LB / amp | LB / amp / ara |
| *E. coli* with plasmid | 1 | 2 | 3 |
| *E. coli* without plasmid plasmid | 4 | 5 | 6 |

16) For each plate, state whether there will be bacterial growth, the type of growth (lawn or colonies), and whether the bacteria will glow. Provide an explanation for your answers.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Plate** | **Growth? (yes or no)** | **Lawn or Colonies?** | **Glow? (yes or no)** | **Explanation** |
| 1 | Yes | Lawn | No | Bacteria will have uninhibited growth on this plate because it contains plain LB agar (bacteria food) |
| 2 | Yes | Colonies | No | Only successfully transformed bacteria will grow on a plate with ampicillin. Bacteria that have not been successfully transformed will die (because they do not have the plasmid / gene for ampicillin resistance), which explains the blanks spots around the colonies. |
| 3 | Yes | Colonies | Yes | Only successfully transformed bacteria will grow on a plate with ampicillin. Bacteria that have not been successfully transformed will die (because they do not have the plasmid / gene for ampicillin resistance), which explains the blanks spots around the colonies. In the presence of the sugar arabinose, the bacteria in the colonies will glow. |
| 4 | Yes | Lawn | No | Bacteria will have uninhibited growth on this plate because it contains plain LB agar |
| 5 | No | N/A | N/A | The bacteria cannot grow on the plate because it contains ampicillin, and the bacteria have not been transformed with the plasmid containing the gene for ampicillin resistance. |
| 6 | No | N/A | N/A | The bacteria cannot grow on the plate because it contains ampicillin, and the bacteria have not been transformed with the plasmid containing the gene for ampicillin resistance. |

17) List the steps involved in creating human insulin protein through using recombinant DNA.

1. Cut the human insulin gene and a bacterial plasmid with the same restriction enzyme
2. Seal the sticky ends of the human insulin gene and the bacterial plasmid together with DNA ligase
3. Induce a bacterium to take in the recombinant plasmid by putting it in a CaCl2 solution and heat-shocking it.
4. Allow the bacterium to transcribe and translate its plasmid DNA as well as the human insulin gene. Extract the human insulin protein that is created.

18) Golden rice is a transgenic plant, meaning it contains a gene from another organism. In this case, it has been given the gene for the creation of beta carotene (vitamin A). How can a bacterium be used as a “vector” to insert the beta carotene gene into the golden rice plant cells?

1. Perform steps 1-3 above using the gene for the creation of beta carotene (vitamin A) and a plasmid from a bacterium that infects plants (ex: *Agrobacterium tumefaciens).*
2. Allow the bacterium to infect a plant cell (in this case a rice cell), and insert its plasmid DNA and the beta carotene synthesis gene into the plant cell’s DNA.